

# Megalin Contributes to Kidney Accumulation and Nephrotoxicity of Colistin

Takahiro Suzuki,<sup>a</sup> Hiroaki Yamaguchi,<sup>a</sup> Jiro Ogura,<sup>a</sup> Masaki Kobayashi,<sup>a</sup> Takehiro Yamada,<sup>b</sup> Ken Iseki<sup>a,b</sup>

Laboratory of Clinical Pharmaceutics & Therapeutics, Division of Pharmasciences, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan<sup>a</sup>; Department of Pharmacy, Hokkaido University Hospital, Sapporo, Japan<sup>b</sup>

Interest has recently been shown again in colistin because of the increased prevalence of infections caused by multidrug-resistant Gram-negative bacteria. Although the potential for nephrotoxicity is a major dose-limiting factor in colistin use, little is known about the mechanisms that underlie colistin-induced nephrotoxicity. In this study, we focused on an endocytosis receptor, megalin, that is expressed in renal proximal tubules, with the aim of clarifying the role of megalin in the kidney accumulation and nephrotoxicity of colistin. We examined the binding of colistin to megalin by using a vesicle assay. The kidney accumulation, urinary excretion, and concentrations in plasma of colistin in megalin-shedding rats were also evaluated. Furthermore, we examined the effect of megalin ligands and a microtubule-depolymerizing agent on colistin-induced nephrotoxicity. We found that cytochrome *c*, a typical megalin ligand, inhibited the binding of colistin to megalin competitively. In megalin-shedding rats, renal proximal tubule colistin accumulation was decreased ( $13.5 \pm 1.6$  and  $21.3 \pm 2.6$   $\mu\text{g}$  in megalin-shedding and control rats, respectively). Coadministration of colistin and cytochrome *c* or albumin fragments resulted in a significant decrease in urinary *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) excretion, a marker of renal tubular damage ( $717.1 \pm 183.9$  mU/day for colistin alone,  $500.8 \pm 102.4$  mU/day for cytochrome *c* with colistin, and  $406.7 \pm 156.7$  mU/day for albumin fragments with colistin). Moreover, coadministration of colistin and colchicine, a microtubule-depolymerizing agent, resulted in a significant decrease in urinary NAG excretion. In conclusion, our results indicate that colistin acts as a megalin ligand and that megalin plays a key role in the accumulation in the kidney and nephrotoxicity of colistin. Megalin ligands may be new targets for the prevention of colistin-induced nephrotoxicity.

Antimicrobial resistance has become a worldwide health care crisis because of the lack of effective treatments and the limited number of antibiotics available for treating potentially lethal pathogens. Infections with multidrug-resistant Gram-negative bacteria have emerged as a major threat to hospitalized patients and have been associated with mortality rates ranging from 30 to 70% (1–5).

Colistin, a cationic cyclic polypeptide antibiotic, was discovered in Japan and developed in the 1950s for the treatment of Gram-negative infections. The mode of action of this antibiotic is disruption of the outer membrane of Gram-negative bacteria, resulting in increased cell permeability with subsequent leakage of cell contents, cell lysis, and cell death (6–8). Colistin is administered as colistimethate sodium (CMS), a prodrug of colistin, and CMS is considered to have few side effects and is converted to colistin by hydrolysis (9–11). Although colistin was being replaced by  $\beta$ -lactam and aminoglycoside antibiotics in the 1970s, interest has recently been shown again in colistin because of the increased prevalence of infections caused by multidrug-resistant Gram-negative bacteria, especially in critically ill patients (11). For patients with severe infections or elderly patients, the potential for colistin-induced nephrotoxicity is a major dose-limiting factor. Results of some studies on colistin-induced nephrotoxicity from the viewpoint of pharmacodynamics have been reported (12–14); however, there are few reports on colistin-induced nephrotoxicity from the viewpoint of pharmacokinetics.

Megalin, which is encoded by a member of the low-density lipoprotein receptor gene family, is a giant membrane glycoprotein of 600 kDa and is highly expressed on the luminal surface of renal proximal tubules (15, 16). Megalin functions as an endocytic receptor and plays a role in the internalization of its ligands. Li-

gands that bind to megalin represent a variety of classes, including aminoglycosides, albumin, aprotinin, and cytochrome *c* (17–20). It has been reported that polymyxin B, which is structurally similar to colistin, inhibited the binding of plasminogen activator inhibitor type 1 (PAI-1) to megalin (21). Since megalin is present in the renal proximal tubule, it is possible that megalin plays a role in the renal handling of colistin. In the present study, we focused on megalin and aimed to determine whether megalin is relevant to the kidney accumulation and nephrotoxicity of colistin.

## MATERIALS AND METHODS

**Chemicals and reagents.** Colistin sulfate, 9-fluorenylmethyl chloroformate, and equine heart cytochrome *c* were purchased from Sigma (St. Louis, MO). Netilmicin and colchicine were purchased from Wako (Osaka, Japan). Maleic acid was purchased from Nacalai Tesque (Kyoto, Japan). Anti-megalin antibody was purchased from Abcam Inc. (Cambridge, MA). All other chemicals were commercially available and of the highest purity possible.

**Animals.** Male Wistar rats, aged 6 weeks, were obtained from Jla (Tokyo, Japan). The rats were housed for 1 to 2 weeks at 23°C and 60%  $\pm$  10% relative humidity with a 12-h light-dark cycle (until they reached a weight of 270 to 330 g). During the period of acclimatization, the rats had free

Received 5 February 2013 Returned for modification 27 March 2013

Accepted 2 October 2013

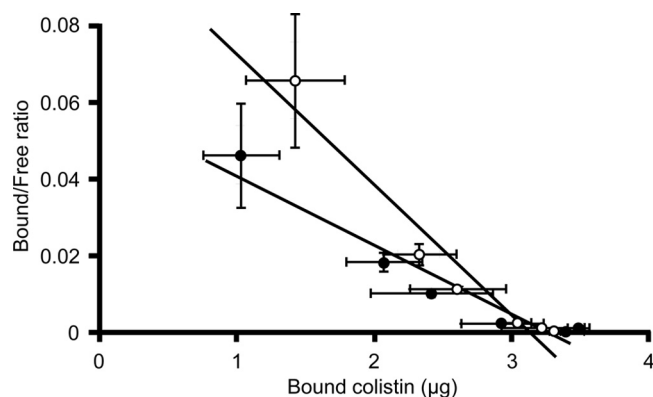
Published ahead of print 7 October 2013

Address correspondence to Ken Iseki, ken-i@pharm.hokudai.ac.jp.

T.S. and H.Y. contributed equally to this work.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.00254-13



**FIG 1** Inhibitory effect of cytochrome *c* on the binding of colistin to megalin. Scatchard plots show the binding of colistin to megalin in the group given colistin alone and in the group given colistin with cytochrome *c*. BBMV's suspended in buffer A containing 0.1, 0.5, 1, 5, 10, or 100  $\mu$ M colistin without or with 1 mM cytochrome *c* were incubated in a membrane suspension at 4°C for 60 min. Open circles represent a Scatchard plot of colistin alone, and solid circles indicate a Scatchard plot of colistin with cytochrome *c*. Each point represents the mean and SD of three measurements.

access to food and water. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the Guide for the Care and Use of Laboratory Animals.

**Western blotting.** Megalin expression was examined as described in a previous report, with some modifications (22). Total protein extracts were prepared from the right kidney cortex. The cortex was homogenized in a lysis buffer (1.0% Triton X-100, 0.1% sodium dodecyl sulfate [SDS], 4.5 M urea). The homogenate was sonicated for 10 s at 4°C and centrifuged at  $4,000 \times g$  for 1 min at 4°C. The samples were then separated by 6% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes (Bio-Rad, Tokyo, Japan) by semidry electroblotting at 25 V for 120 min. The membranes were blocked with a mixture of 1% bovine serum albumin and 1% nonfat dry milk in phosphate-buffered saline (PBS) containing 0.5% Tween 20 (PBS/T) for 1 h. After being washed with PBS/T, the membranes were incubated overnight with a primary antibody specific for megalin. After being washed five times with PBS/T for 10 min each time, the membranes were incubated for 1 h with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody. After being washed five times with PBS/T for 10 min each time, the bound antibody was detected on X-ray film by enhanced chemiluminescence. The relative amounts of bands in each lane were determined densitometrically by using Scion Image.

**Binding assay.** Brush border membrane vesicles (BBMV's) were prepared as described by Biber et al. (23) and stored at  $-80^{\circ}\text{C}$  until use. BBMV's (100  $\mu$ g) were suspended in a buffer consisting of 300 mM mannitol and 10 mM HEPES adjusted to pH 7.5 with KOH (buffer A). The reaction was initiated by the addition of buffer A containing 0.1, 0.5, 1, 5, 10, or 100  $\mu$ M colistin without or with 1 mM cytochrome *c* to the membrane suspension at 4°C. After 60 min, incubation was stopped by diluting the reaction mixture with 1 ml of ice-cold buffer A. The contents of the tube were immediately poured onto Millipore filters (HAWP, 0.45  $\mu$ m,

2.5-cm diameter), and then the filters were washed once with 5 ml of ice-cold buffer A. The amount of bound colistin was measured by high-performance liquid chromatography (HPLC) as previously described (24, 25), and the values of the dissociation constant ( $K_d$ ) and the maximum amounts of binding ( $B_{\text{max}}$ ) were estimated from Scatchard plots.

**Pharmacokinetic studies with rats.** To induce megalin shedding from the renal brush border membrane, rats were given sodium maleate (400 mg/kg, pH 7.4) by intraperitoneal (i.p.) injection (26). Rats injected with the same volume of saline were used as controls. Subsequently, the animals were anesthetized with sodium pentobarbital (30 mg/kg of body weight, i.p. injection), and the femoral artery and vein were cannulated with polyethylene tubing for blood sampling and drug administration, respectively. The urinary bladder was cannulated to collect urine samples. At 2.75 h after maleate injection, colistin (0.5 mg/kg) was administered as a bolus via the femoral vein. Blood samples were collected from the femoral artery at 5, 15, 30, 45, 60, 90, 120, and 180 min after the injection of colistin. At 180 min after the administration of colistin, the kidneys were excised. The excised kidneys were gently washed, weighed, and homogenized in saline. The amounts of colistin in the blood, urine, and kidney homogenate were measured by HPLC (24, 25).

**Effects of megalin ligands on colistin-induced nephrotoxicity and colistin excretion in urine.** Rats were injected intravenously with saline (control), 1.0 mg/kg colistin alone, 1.0 mg/kg colistin with 100 mg/kg cytochrome *c*, or 1.0 mg/kg colistin with 50 mg of albumin fragments (FRALB). FRALB with molecular masses of less than 50 kDa were prepared as described by Vegt et al. (27). The rats were then housed in metabolic cages to collect urine for 24 h with free access to water. *N*-Acetyl- $\beta$ -D-glucosaminidase (NAG) in the urine was selected to evaluate nephrotoxicity (17, 28) and was measured with a commercially available kit (Shionogi and Co., Osaka, Japan).

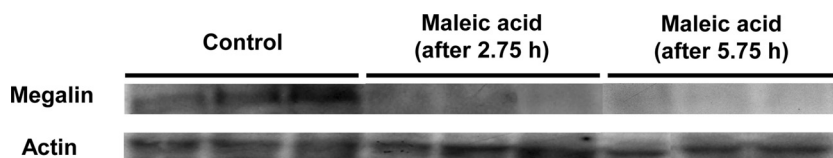
**Effects of colchicine on colistin-induced nephrotoxicity.** Colchicine treatment for megalin internalization from the brush border membrane into intracellular vesicles was carried out as described previously (29). Rats were injected intravenously with 1.0 mg/kg colistin alone or 1.0 mg/kg colistin after the injection of colchicine (3.5 mg/kg, i.p.). Rats were then housed in metabolic cages with free access to water to collect urine for 24 h. NAG in the urine was measured.

**Pharmacokinetic analysis.** A conventional one-compartment model was used to analyze the plasma concentration-time profile of colistin after intravenous administration to rats. The parameters total body clearance ( $\text{CL}_{\text{tot}}$ ), volume of distribution ( $V_d$ ), and half-life ( $t_{1/2}$ ) were calculated by the nonlinear least-squares method. Renal clearance ( $\text{CL}_R$ ) was calculated by dividing the amount of colistin eliminated in urine in 180 min by the area under plasma concentration-time curve for 180 min ( $\text{AUC}_{0-180}$ ), which was calculated by using the linear trapezoidal rule. Nonrenal clearance ( $\text{CL}_{\text{NR}}$ ) was obtained by subtracting the  $\text{CL}_R$  from the  $\text{CL}_{\text{tot}}$ .

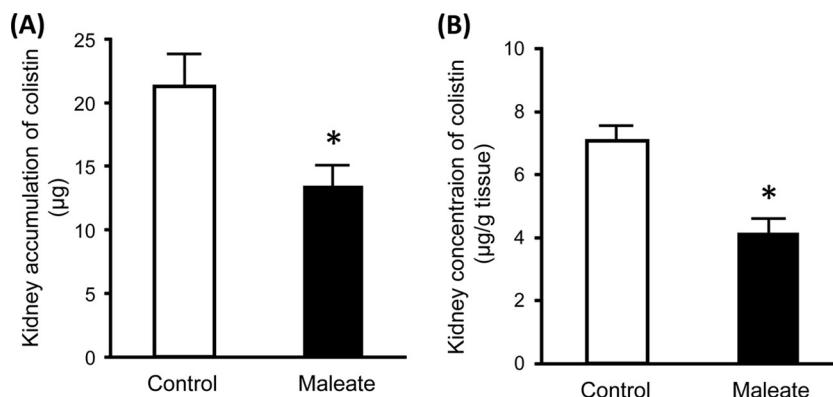
**Statistical analysis.** Values are expressed as means  $\pm$  standard deviations (SD). The statistical significance of differences between mean values was analyzed with the nonpaired *t* test. Multiple comparisons were performed by using Scheffé's test following analysis of variance. Differences were considered significant at a *P* value of  $<0.05$ .

## RESULTS

**Binding assay.** First, we examined whether colistin binds to megalin. Figure 1 shows the inhibitory effect of a megalin ligand, cyto-



**FIG 2** Effect of maleic acid on kidney cortex megalin expression. Western blotting was used to detect megalin in the renal cortices of control rats and rats given an i.p. injection of maleate (400 mg/kg). Renal cortex samples (20  $\mu$ g/lane) were subjected to 6% SDS-PAGE.



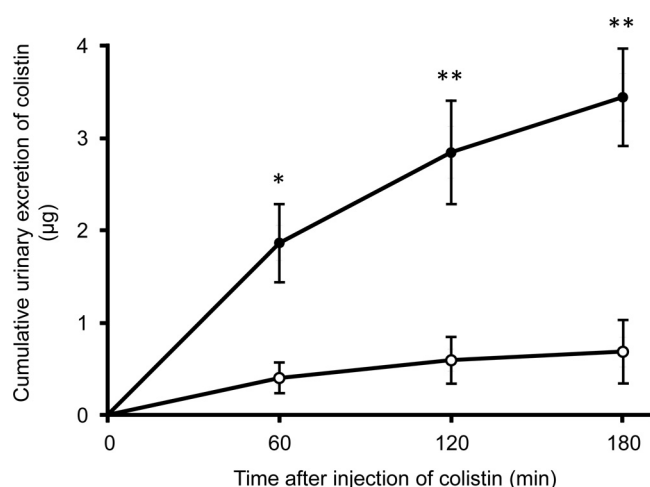
**FIG 3** Kidney accumulation (A) and concentrations (B) of colistin in megalin-shedding rats. At 45 min after the administration of an i.p. injection of maleate (400 mg/kg) or saline (normal), colistin (0.5 mg/kg) was injected intravenously. Kidneys were collected at 180 min after the administration of colistin. Colistin contents in the kidneys (A) were examined by HPLC, and kidney colistin concentrations (B) were calculated by using kidney weight. Each column represents the mean and SD of four measurements. \*,  $P < 0.05$  (versus the control).

chrome *c*, on the binding of colistin to megalin. The apparent  $K_d$  values were  $130.3 \pm 79.0$  nM for colistin alone and  $227.4 \pm 79.4$  nM for colistin with cytochrome *c*. The  $B_{\text{max}}$  values for colistin alone and colistin with cytochrome *c* were  $3.24 \pm 0.22$  and  $3.36 \pm 0.14$  ng, respectively.

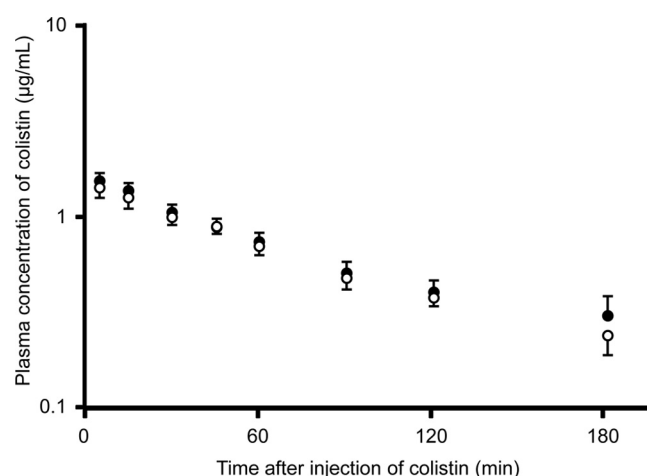
**Effect of megalin shedding on colistin pharmacokinetics.** It has been reported that maleate in rats induces megalin shedding from the brush border membrane in renal proximal tubule cells and increases the urinary excretion of megalin (26, 30). Using this model, we examined the contribution of megalin to colistin pharmacokinetics. As the first step, we confirmed a decrease in megalin in renal proximal tubules caused by the administration of maleate by using Western blot analysis (Fig. 2). We then examined the pharmacokinetics of colistin in control and maleate-treated rats. The amount of colistin that accumulated in the kidneys of megalin-shedding rats was significantly smaller than that in control rats ( $13.5 \pm 1.6$  and  $21.3 \pm 2.6$   $\mu\text{g}$  in megalin-shedding and control

rats, respectively, Fig. 3), and the cumulative amount of colistin in urine was significantly larger in megalin-shedding rats ( $3.4 \pm 0.5$  and  $0.7 \pm 0.3$   $\mu\text{g}$  in megalin-shedding and control rats, respectively, Fig. 4). Figure 5 shows that there was no difference between the plasma colistin concentrations of megalin-shedding and control rats. The pharmacokinetic parameters of colistin are summarized in Table 1.  $\text{CL}_{\text{tot}}$ ,  $t_{1/2}$ , and  $V_d$  were not affected by maleate treatment.  $\text{CL}_R$  was significantly increased in megalin-shedding rats ( $0.070 \pm 0.015$  and  $0.016 \pm 0.007$  ml/min in megalin-shedding and control rats, respectively); however,  $\text{CL}_{\text{NR}}$  was not altered.

**Effects of megalin ligands on colistin-induced nephrotoxicity and colistin excretion in urine.** To reveal the effect of megalin on the nephrotoxicity of colistin, we examined the effects of megalin ligands on colistin-induced nephrotoxicity. In rats injected intravenously with 1.0 mg/kg colistin, the 24-h urinary NAG excretion was three times as high as that in control rats ( $717.1 \pm 183.9$



**FIG 4** Cumulative amounts of colistin in the urine of megalin-shedding rats. The urinary bladders of rats were cannulated, and urine samples were collected at 60, 120, and 180 min after the administration of colistin. Open circles represent control rats, and solid circles represent megalin-shedding rats. Each point represents the mean and SD of four measurements. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  (versus the control).



**FIG 5** Plasma colistin concentrations in megalin-shedding rats. The femoral arteries of rats were cannulated with polyethylene tubing, and blood samples were collected at 5, 15, 30, 45, 60, 90, 120, and 180 min after the injection of colistin. Open circles represent control rats, and solid circles represent megalin-shedding rats. Each point represents the mean and SD of four measurements.

TABLE 1 Colistin pharmacokinetics in megalin-shedding rats<sup>a</sup>

Treatment	<i>t</i> <sub>1/2</sub> (min)	<i>V</i> <sub>d</sub> (ml)	CL <sub>tot</sub> (ml/min)	CL <sub>R</sub> (ml/min)	CL <sub>NR</sub> (ml/min)
Control	32.9 ± 8.0	77.4 ± 12.0	1.63 ± 0.13	0.016 ± 0.007	1.62 ± 0.12
Maleate	34.8 ± 8.4	76.6 ± 3.9	1.54 ± 0.18	0.070 ± 0.015 <sup>b</sup>	1.47 ± 0.17

<sup>a</sup> Colistin pharmacokinetics were analyzed by using a one-compartmental model. Values are means and SDs of four rats.

<sup>b</sup> Statistically significantly different from the control (*P* < 0.01).

and 269.2 ± 40.5 mU/day for colistin alone and the control, respectively, Fig. 6). Coadministration of colistin and a megalin ligand, cytochrome *c* or FRALB, resulted in a significant decrease in urinary NAG excretion (500.8 ± 102.4 and 406.7 ± 156.7 mU/day for cytochrome *c* with colistin and FRALB with colistin, respectively). The administration of cytochrome *c* or FRALB alone did not affect the urinary excretion of NAG (97.4% ± 43.8% and 94.2% ± 27.7% of the control for cytochrome *c* and FRALB, respectively). Also, urinary colistin excretion was higher in the cytochrome *c*-treated (6.2 ± 2.3 μg/day) and FRALB-treated (5.4 ± 0.9 μg/day) groups than in the colistin-alone group (3.7 ± 1.4 μg/day) (Fig. 7).

**Effect of colchicine on colistin-induced nephrotoxicity and colistin excretion in urine.** We examined the effect of colchicine, a microtubule-depolymerizing agent, on colistin-induced nephrotoxicity. Coadministration of colistin and colchicine resulted in a significant decrease in urinary NAG excretion (717.1 ± 183.9 and 362.4 ± 126.7 mU/day for colistin alone and colchicine with colistin, respectively) (Fig. 8).

DISCUSSION

Polymyxin B, which is structurally similar to colistin, is known to inhibit the binding of PAI-1 to megalin (21). However, it is not known whether polymyxins behave as megalin ligands or not. We therefore examined the binding of colistin to megalin by using a vesicle assay. In the presence of cytochrome *c*, the amount of colistin that bound to megalin was decreased. *B*<sub>max</sub> values did not change with or without cytochrome *c* (Fig. 1). These results suggest that cytochrome *c* inhibits the binding of colistin to megalin competitively and that colistin acts as a megalin ligand.

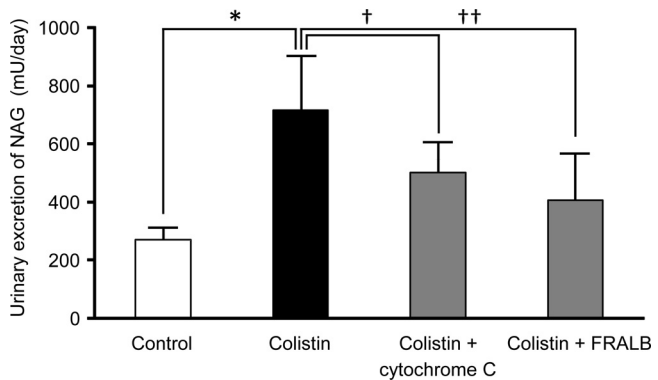


FIG 6 Effects of megalin ligands on urinary NAG excretion. Rats were injected intravenously with saline (control), 1.0 mg/kg colistin alone, 1.0 mg/kg colistin with 100 mg/kg cytochrome *c*, or 1.0 mg/kg colistin with 50 mg FRALB. The rats were housed in metabolic cages to collect urine for 24 h. Each column represents the mean and SD of five measurements. \*, *P* < 0.05 (versus the control); †, *P* < 0.05; ††, *P* < 0.01 (versus colistin alone).

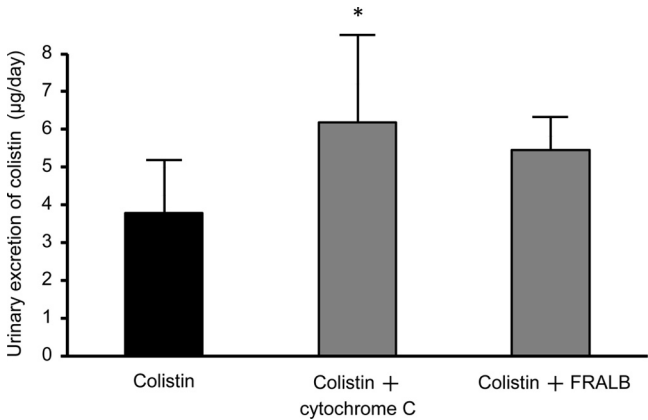


FIG 7 Effects of megalin ligands on urinary colistin excretion. The 24-h urinary colistin excretion of groups treated with 1.0 mg/kg colistin alone, 1.0 mg/kg colistin with 100 mg/kg cytochrome *c*, and 1.0 mg/kg colistin with 50 mg FRALB is shown. The colistin contents of urine samples were examined by HPLC. Each column represents the mean and SD of five measurements. \*, *P* < 0.05 (versus colistin alone).

Megalín is expressed in the renal proximal tubule epithelium (15, 16, 21) and is involved in the binding and endocytosis of polybasic drugs such as aminoglycosides and aprotinin in the renal brush border membrane (17–20). To test whether megalín shedding increases the CL<sub>R</sub> but not the CL<sub>NR</sub> of colistin, the effect of megalín shedding caused by maleate treatment on the pharmacokinetics of colistin was examined. As expected, in megalín-shedding rats, kidney colistin accumulation was lower (Fig. 3) and the cumulative amount of colistin in a 3-h urine sample was larger than that in control rats (Fig. 4). These results suggest that colistin after glomerular filtration is taken up by proximal tubule cells via megalín. However, megalín shedding did not affect the plasma colistin concentration or colistin pharmacokinetic parameters, except for CL<sub>R</sub> (Fig. 5 and Table 1). It has been reported that the CL<sub>R</sub> of colistin is far lower than the expected CL<sub>R</sub> by glomerular filtration (2.3 ml/min/kg), which is calculated as the unbound fraction of colistin (0.44) and the glomerular filtration rate in rats (5.2 ml/min/kg) (31, 32). In our results, although the CL<sub>R</sub> of colis-

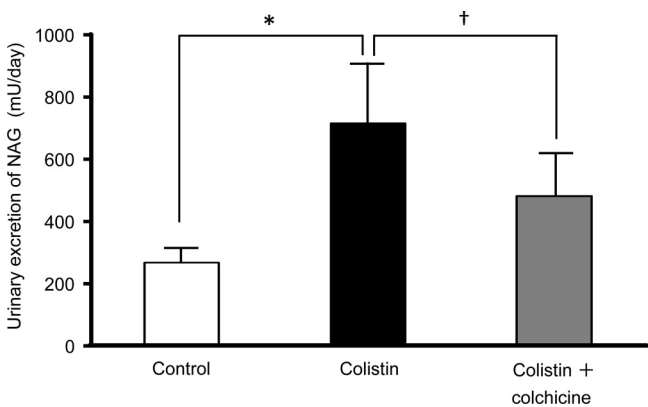


FIG 8 Effect of colchicine on urinary NAG excretion. Rats were injected intravenously with saline (control), 1.0 mg/kg colistin alone, or 1.0 mg/kg colistin with 3.5 mg/kg colchicine. The rats were housed in metabolic cages to collect urine for 24 h. Each column represents the mean and SD of five measurements. \*, *P* < 0.05 (versus the control); †, *P* < 0.05 (versus colistin alone).



tin in maleate-treated rats was 4-fold higher than that in control rats (Table 1), renal tubular reabsorption of colistin via megalin does not account for the considerable extensive reabsorption seen. Therefore, megalin has an important role in colistin accumulation in the kidney but not in the CL<sub>NR</sub>. These results provide the first evidence of colistin accumulation mediated by megalin.

Although colistin has become the “savior” of patients with multidrug-resistant Gram-negative infections, the potential for nephrotoxicity is a major dose-limiting factor (6–8, 11). It has been reported that antioxidant compounds such as melatonin and *N*-acetylcysteine prevent colistin-induced nephrotoxicity (12–14). However, there has been no nephrotoxicity study from the point of view of pharmacokinetics. We focused on the renal disposition of colistin and examined the coadministration of colistin and megalin ligands. First, we examined tail vein injection of 1.0 mg/kg colistin alone and confirmed colistin-induced nephrotoxicity (Fig. 6). We then found that coadministration of colistin with cytochrome *c* and FRALB significantly decreased 24-h urinary NAG excretion by 70 and 56%, respectively (Fig. 6). Although it is possible that these megalin ligands affect the plasma colistin concentration, these results were due to competitive renal colistin uptake because of the increase in the 24-h urinary excretion of colistin (Fig. 7). It is known that megalin depends on microtubules in recycling, and colchicine, the inhibitor of microtubule polymerization, has megalin internalized (29, 33). We examined the effect of colchicine, a microtubule-depolymerizing agent, on colistin-induced nephrotoxicity and found that coadministration of colistin and colchicine resulted in a significant decrease in urinary NAG excretion (Fig. 8). These results support the speculation regarding the mechanism of colistin accumulation in the kidney. In our experiments, we could not completely inhibit colistin uptake into the kidney by using megalin inhibitors. It has been reported that organic cation/carnitine transporter 1 (OCTN1) partially mediates colistin reabsorption after glomerular filtration (32), but whether OCTN1 contributes to colistin nephrotoxicity is unknown. Anyway, we reveal for the first time that nephrotoxicity of colistin is related to megalin. Inhibition of the binding of colistin to megalin may be a new preventive measure against colistin-induced nephrotoxicity, or a novel colistin derivative that is not recognized by megalin may have less nephrotoxicity.

**Conclusion.** We showed that colistin behaves as a megalin ligand *in vitro*. Results obtained by using *in vivo* megalin-shedding model rats suggest that megalin plays an important role in colistin accumulation in the kidney. The results also indicate that megalin is involved in the nephrotoxicity of colistin and that inhibition of the binding of colistin to megalin may be a new preventive measure against nephrotoxicity. Our study provides fundamental information about the appropriate use of colistin.

## ACKNOWLEDGMENT

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## REFERENCES

- Gudiol C, Tubau F, Calatayud L, Garcia-Vidal C, Císnal M, Sánchez-Ortega I, Duarte R, Calvo M, Carratalà J. 2011. Bacteraemia due to multidrug-resistant Gram-negative bacilli in cancer patients: risk factors, antibiotic therapy and outcomes. *J. Antimicrob. Chemother.* 66:657–663.
- Leibovici L, Samra Z, Konigsberger H, Drucker M, Ashkenazi S, Pitlik SD. 1995. Long-term survival following bacteremia or fungemia. *JAMA* 274:807–812.
- Mouloudi E, Protonotariou E, Zagorianou A, Iosifidis E, Karapanagiotou A, Giasnetsova T, Tsioka A, Roilides E, Sofianou D, Gritsi-Gerogianni N. 2010. Bloodstream infections caused by metallo- $\beta$ -lactamase/Klebsiella pneumoniae carbapenemase-producing *K. pneumoniae* among intensive care unit patients in Greece: risk factors for infection and impact of type of resistance on outcomes. *Infect. Control Hosp. Epidemiol.* 31:1250–1256.
- Tamma PD, Cosgrove SE, Maragakis LL. 2012. Combination therapy for treatment of infections with Gram-negative bacteria. *Clin. Microbiol. Rev.* 25:450–470.
- Boyd N, Nailor MD. 2011. Combination antibiotic therapy for empiric and definitive treatment of Gram-negative infections: insights from the Society of Infectious Diseases Pharmacists. *Pharmacotherapy* 31:1073–1084.
- Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K. 2005. Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. *Int. J. Antimicrob. Agents* 25:11–25.
- Falagas ME, Kasiakou SK, Tsiodras S, Michalopoulos A. 2006. The use of intravenous and aerosolized polymyxins for the treatment of infections in critically ill patients: a review of the recent literature. *Clin. Med. Res.* 4:138–146.
- Falagas ME, Kasiakou SK. 2005. Colistin: the revival of polymyxins for the management of multidrug-resistant Gram-negative bacterial infections. *Clin. Infect. Dis.* 40:1333–1341.
- Bergen PJ, Li J, Rayner CR, Nation RL. 2006. Colistin methanesulfonate is an inactive prodrug of colistin against *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 50:1953–1958.
- Kwa AL, Tam VH, Falagas ME. 2008. Polymyxins: a review of the current status including recent developments. *Ann. Acad. Med. Singapore* 37: 870–883.
- Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, Paterson DL. 2006. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *Lancet Infect. Dis.* 6:589–601.
- Ozyilmaz E, Ebinc FA, Derici U, Gulbahar O, Goktas G, Elmas C, Oguzlugen IK, Sindel S. 2011. Could nephrotoxicity due to colistin be ameliorated with the use of *N*-acetylcysteine? *Intensive Care Med.* 37:141–146.
- Yousef JM, Chen G, Hill PA, Nation RL, Li J. 2011. Melatonin attenuates colistin-induced nephrotoxicity in rats. *Antimicrob. Agents Chemother.* 55:4044–4049.
- Yousef JM, Chen G, Hill PA, Nation RL, Li J. 2012. Ascorbic acid protects against the nephrotoxicity and apoptosis caused by colistin and affects its pharmacokinetics. *J. Antimicrob. Chemother.* 67:452–459.
- Marzolo MP, Farfán P. 2011. New insights into the roles of megalin/LRP2 and the regulation of its functional expression. *Biol. Res.* 44:89–105.
- Christensen EI, Verroust PJ, Nielsen R. 2009. Receptor-mediated endocytosis in renal proximal tubule. *Pflügers Arch.* 458:1039–1048.
- Watanabe A, Nagai J, Adachi Y, Katsube T, Kitahara Y, Murakami T, Takano M. 2004. Targeted prevention of renal accumulation and toxicity of gentamicin by aminoglycoside binding receptor antagonists. *J. Control. Release* 95:423–433.
- Cui S, Verroust PJ, Moestrup SK, Christensen EI. 1996. Megalin/gp330 mediates uptake of albumin in renal proximal tubule. *Am. J. Physiol.* 271:F900–F907.
- Orlando RA, Exner M, Czekay RP, Yamazaki H, Saito A, Ullrich R, Kerjaschki D, Farquhar MG. 1997. Identification of the second cluster of ligand-binding repeats in megalin as a site for receptor-ligand interactions. *Proc. Natl. Acad. Sci. U. S. A.* 94:2368–2373.
- Orlando RA, Rader K, Authier F, Yamazaki H, Posner BI, Bergeron JJ, Farquhar MG. 1998. Megalin is an endocytic receptor for insulin. *J. Am. Soc. Nephrol.* 9:1759–1766.
- Moestrup SK, Cui S, Vorum H, Bregengård C, Bjørn SE, Norris K, Gliemann J, Christensen EI. 1995. Evidence that epithelial glycoprotein 330/megalín mediates uptake of polybasic drugs. *J. Clin. Invest.* 96:1404–1413.
- Komazawa H, Yamaguchi H, Hidaka K, Ogura J, Kobayashi M, Iseki K. 2013. Renal uptake of substrates for organic anion transporters Oat1 and Oat3 and organic cation transporters Oct1 and Oct2 is altered in rats with adenine-induced chronic renal failure. *J. Pharm. Sci.* 102:1086–1094.
- Biber J, Stieger B, Stange G, Murer H. 2007. Isolation of renal proximal tubular brush-border membranes. *Nat. Protoc.* 2:1356–1359.
- Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K, Johnson DW.

2001. A simple method for the assay of colistin in human plasma, using pre-column derivatization with 9-fluorenylmethyl chloroformate in solid-phase extraction cartridges and reversed-phase high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* 761:167–175.
25. Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K. 2003. Stability of colistin and colistin methanesulfonate in aqueous media and plasma as determined by high-performance liquid chromatography. *Antimicrob. Agents Chemother.* 47:1364–1370.
26. Nagai J, Tanaka H, Nakanishi N, Murakami T, Takano M. 2001. Role of megalin in renal handling of aminoglycosides. *Am. J. Physiol. Renal Physiol.* 281:F337–F344.
27. Vegt E, van Eerd JE, Eek A, Oyen WJ, Wetzels JF, de Jong M, Russel FG, Masereeuw R, Gotthardt M, Boerman OC. 2008. Reducing renal uptake of radiolabeled peptides using albumin fragments. *J. Nucl. Med.* 49:1506–1511.
28. Vaidya VS, Ozer JS, Dieterle F, Collings FB, Ramirez V, Troth S, Muniappa N, Thudium D, Gerhold D, Holder DJ, Bobadilla NA, Marrer E, Perentes E, Cordier A, Vonderscher J, Maurer G, Goering PL, Sistare FD, Bonventre JV. 2010. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. *Nat. Biotechnol.* 28:478–485.
29. Baus M, Medjugorac-Popovski M, Brown D, Sabolic I. 2000. In colchicine-treated rats, cellular distribution of AQP-1 in convoluted and straight proximal tubule segments is differently affected. *Pflugers Arch.* 439:321–330.
30. Bergeron M, Mayers P, Brown D. 1996. Specific effect of maleate on an apical membrane glycoprotein (gp330) in proximal tubule of rat kidneys. *Am. J. Physiol.* 271:F908–F916.
31. Li J, Milne RW, Nation RL, Turnidge JD, Smeaton TC, Coulthard K. 2003. Use of high-performance liquid chromatography to study the pharmacokinetics of colistin sulfate in rats following intravenous administration. *Antimicrob. Agents Chemother.* 47:1766–1770.
32. Ma Z, Wang J, Nation RL, Li J, Turnidge JD, Coulthard K, Milne RW. 2009. Renal disposition of colistin in the isolated perfused rat kidney. *Antimicrob. Agents Chemother.* 53:2857–2864.
33. Gutmann EJ, Niles JL, McCluskey RT, Brown D. 1989. Colchicine-induced redistribution of an apical membrane glycoprotein (gp330) in proximal tubules. *Am. J. Physiol.* 257:C397–407.